Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 7(4); 216-222

ISSN: 0976 822X

Research Article

Evaluation of Newly Formulated Polyherbal Antidiabetic Tablets in Alloxan Induced Diabetes Mellitus in Rats

Manandhar Suman, Shivalinge Gowda K P*

Department of Pharmacology, PES College of Pharmacy, Hanumanthnagar, Bangalore-560050, Karnataka, India.

Available Online:15th July, 2016

ABSTRACT

In this study rats were divided into 5 groups (n=6). Group I normal control, II received (Polyherbal Antidiabetic tablet) PHADT (400 mg /kg b.w p.o, III alloxan (120mg/kg b.w i.p) treated diabetic rats, IV alloxan +PHADT, V alloxan+Acarbose (20mg/kg bw p.o).The treatment is made for 15 days. The body weight, feed intake was measured daily, blood sugar level measured every 5th day. OGTT was estimated on 15th day. On 16th day the blood was withdrawn from retoorbital plexus and the serum was used for the lipid profile estimation. After scarification under overdose of ketamine, the liver and skeletal muscle glycogen was measured. The alloxan induced diabetic rats treated with PHADT, Acarbose showed significantly increased body weight, decreased feed intake, decreased blood sugar level, high postprandial glucose clearance and decreased serum lipid profile, increased skeletal muscle glycogen content when compared to alloxan induced diabetic rats (Group III). Significant regeneration of pancreatic β cells was observed in diabetic rats treated with PHADT, Acarbose. From this study, it can be concluded that Poly Herbal Anti Diabetic Tablet(PHADT) has anti-hyperglycemic activity as well as anti hyperlipidemic activity.

Keywords: Alloxan; PHADT; serum lipid profile; Acarbose.

INTRODUCTION

Diabetes Mellitus(DM) consists of a group of disorders characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease1. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. Approximately 1.25 million American children and adults have type 1 diabetes[2]. Prevalence of diabetes is increasing in the Asian countries and it contributes to more than 60% of the world's diabetic population. Asians have large genetic and ethnic predeposition for diabetes hence develop diabetes at a younger age and at a lower body mass index and waist circumference when compared with the Western population. The prevalence among adults aged 20-70 years is expected to rise from 285 million in 2010 to 438 million by the year 2030[3]. At present, available therapies for diabetes mellitus include insulin and many oral hypoglycemic agents, such as biguanides and sulfonylureas. However, these drugs used in the treatment of diabetes mellitus possess a number of limitations, such as adverse effects and high rates of secondary failure. The plant kingdom holds great potential to meet this need. However, scientific testing and validation of the efficacy of most medicinal plants in alleviating DM1 and DM2 is rare. Thus, we have limited knowledge of their safety and efficacy, as most of the data is based on information obtained from traditional medicinal plant practitioners[4]. The recent study shows that 30% of diabetic people use complementary and alternative method of treatment. Herbal medicine is the oldest known medical healthcare and is being used since centuries by many cultures. In the present study the Antihyperglycemic and Antihyperlipidaemic activity of the Polyherbal combination was investigated and compared with the standard antihyperglycemic drug.

MATERIALS AND METHOD

Animals

Adult male Wistar rats (180 – 250 g) were procured from authenticated supplier, Invivo Bioscience, Bangalore. All animals were housed under standard laboratory conditions, maintained on a 12 h light: 12 h dark cycle and food and water were provided ad libitum. Animals were

Figure 1: PHADT tablet.

*Author for Correspondence

Table 1: Herbal Powder mix composition.

S. No.	Botanical name	Family	Parts used	Common Name	Quantity
1.	Gymnema sylvestre	Asclepiadaceae	Leaves	Gudmar	30 gm
2.	Momordica charantia	Cucurbitaceae	Seeds	Karela	10 gm
3.	Phyllanthus amarus	Euphorbiaceae	Fruits	Amla	10 gm
4.	Ocimum Sanctum	Lamiaceae	Leaves	Tulasi	5 gm
5.	Trigonella foenum graecum	Fabaceae	Seeds	Methi	5 gm
6.	Allium sativum	Amaryllidaceae	Bulb	Garlic	5 gm

Table 2: Composition of each tablet.

S. No.	Ingredients	Quantity taken
1.	Herbal powder mix	400mg
2.	Microcrystalline cellulose	380mg
3.	Crospovidone	15mg
4.	Magnesium stearate	2mg
5.	Aerosil	3mg

acclimatized for 7 days to laboratory conditions before the test. The experimental protocols were approved by the Institutional Animal Ethics Committee (Ref No: PESCP/IAEC/06/2014.Date:13-12-2014) and conducted according to CPCSEA guidelines (CPCSEA Reg. no:600/PO/Ere/S/02/CPCSEA), Govt. of India. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Acute Oral Toxicity Study

Acute oral toxicity study was performed for newly developed herbal formulation according to the toxic class method 425 as per OECD (Organization for Economic Co-operation and Development) guidelines. Female Wistar rats (6) weighing between 150-180 g were used for acute toxicity study to determine Lethal Dose, 50% (LD50) of extract. The rats were given dose p.o. up to 4000mg/kg and no mortality was observed for this dose. The animals which were administered with the drug-treated animals were observed carefully for toxicity signs and mortality. 400mg/kg was selected as the suitable dose for this study.

Collection of plant material

The fresh plants viz. Gudmar, Amla, were collected from GKVK (Gandhi Krishi Vignan Kendra), University of Agricultural Science, Karnataka, India and Tulasi, Methi seeds, Karela, Garlic were obtained from authorized supplier, Bangalore in the month of December 2015. The plant was identified and authenticated by Dr. KP Sreenath, Taxonomist, Department Botany, Bangalore University, Bengaluru, India and voucher specimen of the plant was kept in the college herbarium.

Tablet preparation and Evaluation

All the individual herbal drugs were dried using hot air oven at 40^0C for 24 h. The individual herbal drugs were then crushed using grinder and passed through mesh No.40. The individual herbal drugs were then weighed as per the quantity required on digital precision balance. The herbal drugs were mixed geometrically using blender. The powder was tested and evaluated by pre-formulation studies: angle of repose, bulk density, tapped density, compressibility index, and Hausner's ratio before tablet compression. Then, 400mg of the mixed powder was weighed and excipients were added and compressed into tablets using a single punch tableting machine. After that, the tablets were tested for the physical properties: weight variation, friability, tablets thickness, tablets hardness, and disintegration time.

Induction of diabetes

After 12 h fasting of rats, stable diabetes was induced by a single intraperitoneal injection of Alloxan (120 mg/kg)[5] dissolved in 0.1 mol/L sodium citrate buffer (pH 4.5) to Wistar rats; and control group rats received only citrate buffer. Rats then was given standard diet (20% glucose solution for 12 h to prevent initial drug-induced hypoglycemic mortality) and water *ad libitum* for 1 week. After 4 days[6], only rats with FPG over 11.1 mMol (≥200mg/dl) was considered to be qualified diabetic rats and was used for the experiment.

Pharmacological Activities

Animals were divided into 5 groups of 6 rats in each group. Group 1 (normal) received drinking water throughout the course till 15 days. Group 2 received PHADT Tablet(400mg/kg) p.o. for 15 days. Group 3 received Alloxan[5] (120 mg/kg) i.p. 4 days[6] before the study. Group 4 received Herbal Tab (400 mg/kg) p.o. for the duration of 15 days as well as Alloxan[7] (120 mg/kg) i.p. 4 days before the study. Group 5 received STD (Acarbose) 20mg/kg p.o. for 15 days as well as Alloxan (120 mg/kg) i.p. 4 days before the study. Fasting blood samples[5] were collected from the tail vein for blood glucose estimation on 0, 5, 10 and 15th day using one touch Glucometer. The food and water intake was monitored daily for each rat, and the periodical body weight difference of the individual animals was also measured during 15 days of the experimental period. Oral Glucose tolerance test was carried out after 15 days[4].

Biochemical analysis

At the end of the experimental period, overnight fasted animals were sacrificed by cervical decapitation under light ether anesthesia and blood was collected, serum was separated by centrifuging at 3,000 rpm for 10 min. The serum was used for the assay of the biochemical parameters such as total cholesterol[8] (TC), High-density lipoprotein-cholesterol[9] (HDL-C), triglycerides[10] using the diagnostic kits. Low-density lipoprotein-cholesterol (LDL-C)[11] cholesterol VLDL[12] were calculated by using Friedewald's formula. After the biochemical estimations animal was euthanized by overdose of Ketamine anesthesia and liver and skeletal tissue was used for the Glycogen level estimation by using anthrone reagent[13].

Histopathological investigations

The dissected samples of pancreas from each group of diabetic animals were collected in 10% formalin-saline

Table 3: Table showing the different preformulation parameters of the powder mix. (PHADT)

S. No.	Parameters	Values
1.	Angle of repose	29.94±0.93°
2.	Loose Bulk density(LBD)	0.24±0.0066g/ml
3.	Tapped bulk density(TBD)	0.28±0.0057 g/ml
4.	Carr's Index	14.28
5.	Hausner's ratio	1.166

Table 4: Table showing the different post formulation parameters of the tablets.

S. No.	Parameters	Values
1.	% Weight variation	0.859±0.148
2.	Hardness of tablet	2.84±0.23
3.	Friability test	0.362±0.028
4.	Disintegration test	5.16±0.119

solution and stained with hematoxylin and eosin for preparation of section using a microtome and histopathological studies were carried out.

Statistical analysis

Values reported are mean ± standard error. The statistical analysis was carried out using analysis of variance, followed by Bonferroni method of Statistics using the Graph pad prism statistical program. With all analyses, an associated probability (p value) of less than 5%($P<0.05$) was considered significant.

RESULTS

Acute toxicity studies

No toxic symptoms were observed after administration of different dose levels of extract up to a maximum of 4000 mg/kg p.o. according to OECD guideline 425. In addition to this, a dose of 5000 mg/kg dose was administered to a group of animals and symptoms like dyspnea were identified. Hence, the one tenth of safe, tolerable dose was used as a therapeutic dose for further pharmacological study.

Pre formulation parameters

The individual herbal samples were powdered and then the powder was analyzed for the pre- formulation parameters- Angle of repose, Loose bulk density, tapped bulk density, Carr's Index, Hausner's ratio and tabulated in the table.

Post formulation parameters

Color and Appearance

Tablets were light green in appearance with good shape and appearance.

Body Weight

The diabetic rats treated with PHADT tabs have shown significant increase (170.33±4.11) in the body weight when compared to diabetic control (Group III) rats. The diabetic rats treated with Acarbose have also shown significant increase (164.33±8.84) in the body weight when it is compared to Group III (Diabetic control rats).

Feed Intake

Variations in the feed intake in and between various groups during the time period of the study was noted which shows that the change in the feed intake of normal control group animals is stable through the study period. (Decrease in the feed intake on day 0 of normal rats is because of the acclimation condition which later on showed improvement) While the diabetic control group during this study period of 15 days showed significantly less increase in feed intake from day 0 to 15th day. Acarbose (20mg/kg) and PHADT Herbal tablet treated group has shown increase in the feed intake compared to diabetic control group during the entire study period.

Antihyperglycemic activity of PHADT in experimentally induced diabetic rats

The Group I and II rats have shown normal blood sugar level in the beginning day but group III (Diabetic Control), GR IV and Gr V have shown increased blood sugar level (392.8, 359.8, 361.7mg/dl.) But on 15th day, the diabetic control rats (Group III) have shown significant increase (343.5 ± 20.14 mg/dl) in blood sugar level when compared to normal control group (137.3 ± 6.24). The group II rats have not shown any significant difference (147.5 ± 3.51mg/dl) in blood sugar level when compared to Gr I. This shows the Poly Herbal Antidiabetic Tablet does not have hypoglycemic effect. GR IV diabetic rats treated with PHADT tab have shown significant reduction (266.2 ± 15.54**b) in blood sugar level when compared to gr III diabetic rats. GR V diabetic rats administered with Acarbose also showed significant decrease in blood sugar level (259.3 ± 15.1***b) when compared to gr III (Diabetic Control rat).

Effect of PHADT on Glucose tolerance

The oral glucose tolerance test was carried on different groups of rats. The rats of Group I showed significant rise in the blood sugar level at 1,2 and 3h after the oral glucose administration. From oral glucose tolerance test (OGTT)

Table 5: Table showing body weights of Normal, PHADT treated, Acarbose treated and Diabetic rats.

S. No.	Group	Body weight(g)			
		0 Day	5th Day	10th Day	15th Day
1.	Normal Control	144.33 ± 3.63	172.33±3.15	176.00±3.89	179.33±1.90
2.	PHADT treated	149.66 ± 4.17	153.33±8.75	160.66±7.75	171.00±4.40
3.	Diabetic Control	148.66±7.31	138.66±5.64	147.600±4.19	146.00±4.04***^{a}
4.	PHADT treated +Alloxan	165.66±2.98	164.33±4.17	166.00±4.44	170.33±4.11**
5.	Acarbose + Alloxan	145.66±8.13	162.66±9.18	164.66±8.41	164.33±8.84**

Each values represents the Mean ± SEM (n = 6) done by one-way ANOVA followed by Bonferroni Multiple comparison method of Statistics. Where, ***a $p<0.0001$, compared with corresponding values for normal group; **b $p<0.001$, compared with corresponding values for Alloxan treated group.

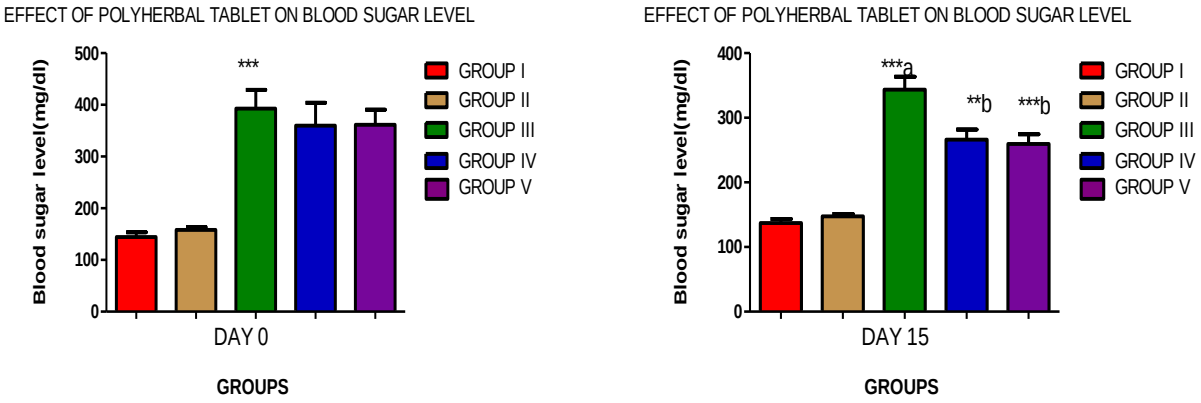

Figure 2a: Effects of Polyherbal tablet (PHADT) on blood sugar in Alloxan treated rats.

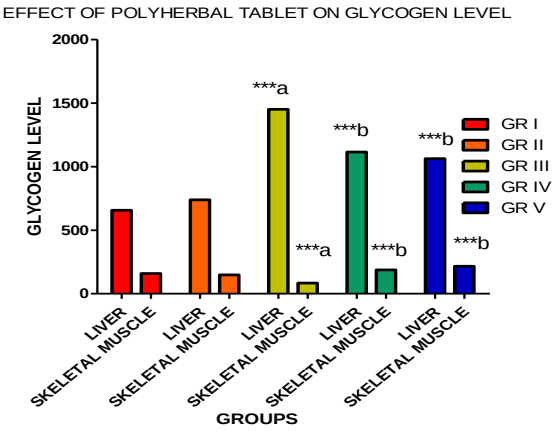

Figure 2b: Effects of Polyherbal tablet (PHADT) on Glycogen level in Alloxan treated rats.

Table 6: Table showing feed intake of Normal, PHADT treated, Acarbose treated and Diabetic rats.

Group	Feed Intake(g)			
	Day 0	Day 5	Day 10	Day 15
I	8	14	16	15.3
II	4.66	7.66	10	10.3
III	6.33	7.66	7.53	7.66
IV	7	7.33	8.66	8.93
V	8.66	10	10.33	12.33

data, it was observed that rats treated with PHADT tablet or 20 mg/kg acarbose showed significantly improved clearance of blood glucose at each time point monitored versus vehicle treated diabetic rat.

Effect of PHADT on lipid profile in Alloxan induced diabetic rats

The concentration of triglycerides and cholesterol were significantly increased in Group III diabetic animals when compared ($P < 0.001$,) to drug-treated Group IV and Group V animals. The increased levels of cholesterol and triglycerides were brought back to near normal by the treatment with PHADT and Acarbose. This observed restoration of the STZ evoked changes in the serum lipid profile shows the protective nature of PHADT. In the present investigation, there is a considerable increase in the HDL-C level ($P < 0.05$) in the animals which received the test drugs when compared to control animals.

Effect of PHADT on Glycogen level in Alloxan induced diabetic rats

Liver Glycogen level

As per the data in the table, it is seen that the liver Glycogen level has increased in Alloxan treated group of rats (1450 ± 2.638) as compared to the Liver Glycogen level in normal Control group of rats (657.0 ± 13.70).GR IV diabetic rats treated with PHADT tab have shown significant decrease (1115 ± 2.280***) in Liver Glycogen level when compared to GR III Diabetic control rats.The Acarbose administered diabetic rats have shown significant decrease in Liver Glycogen level (1063 ± 3.559***) when compared to GrIII Diabetic control rats.

Muscle Glycogen level

As per the data in the table, it is seen that the Skeletal Muscle Glycogen level has decreased in Alloxan treated group of rats (83.62 ± 1.245) as compared to the Skeletal Muscle Liver Glycogen level in normal Control group of rats (158.6 ± 1.112).GR IV diabetic rats treated with PHADT tab have shown significant increase (187.7 ± 1.210***) in Skeletal Muscle Glycogen level when compared to GR III Diabetic control rats.The Acarbose administered diabetic rats have shown significant increase in Skeletal Muscle Glycogen level (216.5 ± 1.849***) when compared to GrIII Diabetic control rats.

Histopathology of Pancreas

Normal Control rats showed normal distribution of acini, delicate collagen fibers around islands of Langerhans.

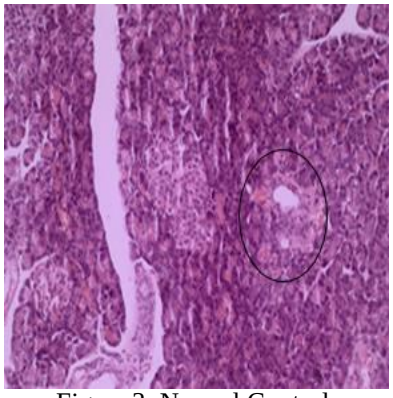
Figure 3: Normal Control.

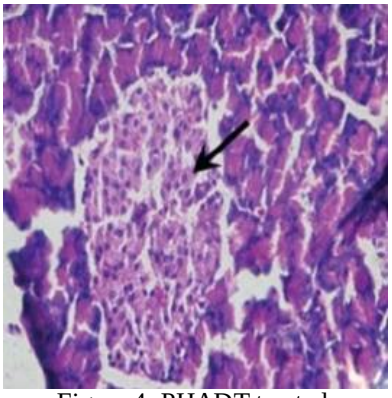
Figure 4: PHADT treated.

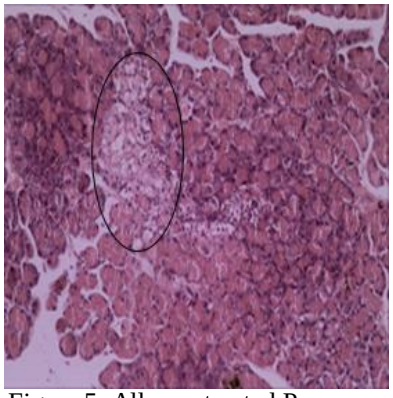
Figure 5: Alloxan treated Pancreas.

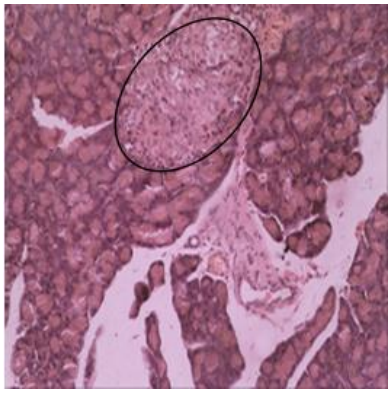
Figure 6: Alloxan +PHADT.

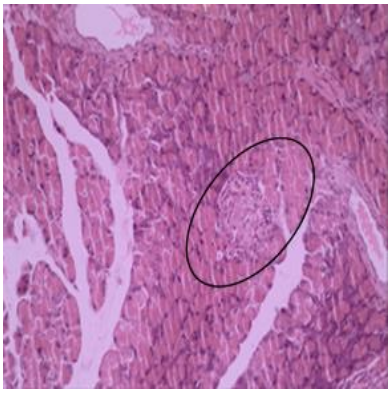
Figure 7: Alloxan +standard.

Table 7: Table showing blood sugar levels of Normal, PHADT treated, Acarbose treated and Diabetic rats.

Group	Blood sugar level (mg/dl)			
	0 Day	5 Day	10 Day	15 Day
I	144.3 ± 9.40	138.8 ± 6.29	137.5 ± 5.95	137.3 ± 6.24
II	158 ± 5.74	154.5 ± 4.14	153.2 ± 5.23	147.5 ± 3.51
III	392.8 ± 35.97***a	375.8 ± 28.62	355.3 ± 22.34	343.5 ± 20.14***a
IV	359.8 ± 44.03	322.8 ± 29.49	290 ± 22.58	266.2 ± 15.54**b
V	361.7 ± 28.98	341 ± 30.27	288.8 ± 22.33	259.3 ± 15.1***b

Each values represents the Mean ± SEM (n = 6) done by one-way ANOVA followed by Bonferroni Multiple comparison method of Statistics. Where, ***a $p<0.0001$, compared with corresponding values for normal group; **b $p<0.001$, ***b $p<0.0001$ compared with corresponding values for Alloxan treated group.

Table 8: Table showing lipid profile of Normal, PHADT treated, Acarbose treated and Diabetic rats.

Group	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	VLDL cholesterol(mg/dl)	LDL cholesterol(mg/dl)	Triglycerides(mg /dl)
I	77.28 ± 4.27	5.94 ± 0.45	5.98 ± 0.46	67.99 ± 3.27	30.37 ± 2.23
II	87.33±2.80***a	9.325 ± 0.6*a	1.86± 0.12**a	76.14± 2.65***a	14.30 ± 0.73***a
III	154.70 ± 3.57***a	1.36 ± 0.026**a	11.58 ± 0.28***a	141.2 ± 3.21***a	57.43 ± 1.47***a
IV	113.6 ± 0.98***b	18.27 ± 0.71***b	4.04 ± 0.15***b	92.08 ± 0.40***b	20.93 ± 0.65***b
V	93.43 ± 0.83***b	40.70 ± 1.67***b	2.07 ± 0.085***b	50.35 ± 2.06***b	9.38 ± 0.38***b

Each bar represents the Mean ± SEM (n = 6) done by one-way ANOVA followed by Bonferroni Multiple comparison method of Statistics. *a $p<0.05$; **a $p<0.001$; *** a$p<0.0001$; compared with corresponding values for Control group (Group I). ***b $p<0.0001$compared with corresponding values for Diabetic Control group (Group III).

Minimal vacuolar changes with islet cell degeneration, atrophy in the islands of Langerhans cells, dense collagen fibers around the acini was seen in Alloxan treated rats. Minimal islet cell regeneration, few collagen fibers around the islets was seen in PHADT and Acarbose treated diabetic rats.
treated pancreas

DISCUSSION

Alloxan was used to induce diabetes which showed severe hyperglycemia as well as metabolic stress due to progressive oxidative damage interrelated with a decrease in endogenous insulin secretion and release. Regenerative pancreatic β-cells can be formed by neogenesis or by replication of the preexisting differentiated cells; since

Table 9: Table showing Glycogen levels of Normal, PHADT treated, Acarbose treated and Diabetic rats.

Group	Treatment	GLYCOGEN LEVEL (mg. of glycogen per g. of tissue)	
		Liver	Skeletal muscle
I	Normal	657.0 ± 13.70	158.6 ± 1.11
II	PHADT tablet Treated	738.51± 3.69	148.8 ± 1.89
III	Alloxan treated	1450 ± 2.63***a	83.62 ± 1.24***a
IV	Alloxan + PHADT tab	1115 ± 2.28***b	187.7 ± 1.21***b
V	Alloxan + Acarbose	1063 ± 3.55***b	216.5 ± 1.84***b

Each bar represents the Mean ± SEM (n = 6) done by one-way ANOVA followed by Bonferroni Multiple comparison method of Statistics. *** a $p<0.0001$; compared with corresponding values for Control group (Group I). ***b $p<0.0001$, compared with corresponding values for Diabetic Control group (Group III).

other medicinal plants have shown β-cell regenerative potential, it is possible that the PHADT were also responsible for the proliferation of β-cells and the recovery of normal pancreatic morphology. Studies show that treatment with phytonutrients might be an effective strategy for reducing diabetes complications by influencing glucose metabolism and homeostasis by mechanisms such as modulation of glucose output from liver, inhibition of carbohydrate digestion and regulating the glucose metabolizing enzymes. In this study the group of diabetic rats showed progressive and significant loss in the body weight throughout the study period. This may be due to insufficient insulin that prevents the body from getting glucose from the blood into the body's cells to use as energy. When this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight. The diabetic rats treated with PHADT have shown significant increase in the body weight when compared to diabetic control (Group III) rats. This may be due to the antidiabetic activity of PHADT which may stimulate the insulin release in rats that leads to the utilization of glucose from blood and prevention of burning of fat and muscle.

In uncontrolled diabetes where blood glucose levels remain abnormally high (hyperglycemia), glucose from the blood cannot enter the cells - due to either a lack of insulin or insulin resistance - so the body can't convert the food into energy. This lack of energy causes an increase in hunger.120In the present study, the diabetic group of rats showed significant increase in the feed intake during the study period. Whereas, the PHADT, Glibenclamide and Acarbose treated rats showed slight reduction in the amount of feed intake. GR IV diabetic rats treated with PHADT tab have shown significant reduction in blood sugar level when compared to gr III diabetic rats. It could be speculated that PHADT might have exerted anti-hyperglycemic effect on diabetic rats by enhancing the insulin secretion. Plants in the PHADT may act on blood glucose through different mechanisms, some of them may have insulin-like substances, some may inhibit insulin activity, and others may increase β cells in the pancreas by activating the regeneration of these cells. Repeated administration of PHADT significantly decreased hypertriglyceridemia and hypercholesterolemia. The observed antihyperlipidemic effect of PHADT may be due to decreased cholesterogenesis and fatty acid synthesis through inhibition of pancreatic cholesterol esterase and pancreatic lipase inhibition effect, respectively[1,2,4]. Glucose homeostasis is mainly regulated by the liver and skeletal muscle. Most glucose disposal occurs in the liver and skeletal muscle, with glycogen as the primary intracellular form of storable glucose(Saltiel,2001). The glycogen levels in various tissues area is direct reflection of insulin sensitivity, as insulin promotes intracellular glycogen deposition. The Skeletal Muscle Glycogen level in diabetic rats has decreased as compared to the normal rats. This is due to Insulin resistance in skeletal muscle which is manifested by decreased insulin-stimulated glucose uptake and results from impaired insulin signaling and multiple post-receptor intracellular defects including impaired glucose transport, glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis[1,2,6].

CONCLUSION

From this study, it can be concluded that Poly Herbal Anti Diabetic Tablet(PHADT) has anti-hyperglycemic activity as it reduced blood glucose level, increased body weight, decreased feed intake in Alloxan induced diabetic rats. In addition, PHADT also have shown significant reduction in the serum cholesterol, Triglyceride, LDL, VLDL level and increased HDL level in diabetic rats which shows that PHADT also possesses Anti hyperlipidemic activity. Furthermore, the reduction in the blood sugar after glucose administration in OGTT test in PHADT treated rats showed that it acts as potent postprandial antihyperglycemic agent. But the exact mechanism behind its activity is not well known. Hence, further more investigation is required for proper identification of mechanism involved.

REFERENCES

1. Laurence B, Keith P, Donald B, Lain B.Goodman and Gilman's Manual of pharmacology and therapeutics.11th ed. Mc Graw Hills; 2006.
2. National Diabetes Statistics Report, 2014, American Diabetes Association.
3. Ramachandran A, Snehalatha C, Shetty A, Nanditha A. Trends in prevalence of diabetes in Asian countries. World J Diabetes. 2012; 3(6): 110-7.
4. Gupta RK, Kesari AN, Murthy PS, Chandra R., Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals. J Ethnopharmacol. 2005; 99: 75–81.
5. Dholi SK, Raparla R, Mankala SK, Nagappan K. In-vivo Antidiabetic evaluation of Neem leaf extract in alloxan induced rats. JAPS. 2011;01(04):100-105.

6. Yimam M, Zhao J, Corneliusen B, Pantier M, Brownell L, Qi J. Blood glucose lowering activity of aloe based composition, UP780, in alloxan induced insulin dependent mouse diabetes model. Diabetol Metab Syndr.2014; 6: 61-8.
7. Xiong WT, Gu Li, Wang C, Sun HX, Xin Liu. Anti-hyperglycemic and Hyperlipidaemic effects of *Cistanche tubulosa* in type2diabetic db/db mice. J Ethnopharmacol. 2013; 150: 935–45.
8. Cholesterol [package insert]. Karasek(CZ): Erba Mannheim; 2013.
9. HDL Cholesterol [package insert]. Karasek(CZ): Erba Mannheim; 2013.
10. Triglyceride [package insert]. Karasek(CZ): Erba Mannheim; 2014.
11. Fukuyama N, Homma K, Wakana N, Kudo K, Suyama A, et al. Validation of the Friedwalds equation for evaluation of Plasma LDL-Cholesterol. J. Clin. Biochem. Nutr. 2008(July); 43:1–5.
12. Sahu S, Chawla R, Uppal B. Comparison of two methods of estimation of low density lipoprotein cholesterol, the direct versus friedewald estimation. IJCB,2005; 20(2): 54-61.
13. Carroll N, Longley R, Roe J. The determination of glycogen in liver and Muscle by use of anthrone reagent. J. Biol. Chem. 1956; 220: 583-93.